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Review

The role of complement in Alzheimer's disease pathology

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Abstract

Complement proteins are integral components of amyloid plaques and cerebral vascular amyloid in Alzheimer brains. They can be found at the earliest stages of amyloid deposition and their activation coincides with the clinical expression of Alzheimer's dementia. This review will examine the origins of complement in the brain and the role of β-amyloid peptide (Aβ) in complement activation in Alzheimer's disease, an event that might serve as a nidus of chronic inflammation. Pharmacology therapies that may serve to inhibit Aβ-mediated complement activation will also be discussed.

Keywords: β-Amyloid peptide; Inflammation; Neurodegeneration; Microglia

1. The natural history of Alzheimer's disease

A multitude of factors (Fig. 1) contribute to a reproducible set of neurochemical (e.g. cholinergic deficits) and neuropathological (e.g. neurofibrillary tangles and amyloid plaques) changes that collectively lead to Alzheimer's disease (AD) dementia [1–10]. Neurofibrillary tangles (NFTs) are fibrillar aggregates of the hyperphosphorylated cytoskeletal protein tau, which is associated with microtubules. NFTs may be found both intracellularly and extracellularly. The amyloid plaques appear to result from the overproduction and/or reduced clearance of the β-amyloid peptide (Aβ). Aβ accumulates mainly as fibrillar deposits in the brain parenchyma and in the leptomeningeal blood vessels of the hippocampus and cortex. Aβ is proteolytically derived from the β-amyloid precursor protein (βAPP) by, as yet, unidentified proteases, termed β-secretase(s) and γ-secretase(s). These secretases respectively cleave at the NH_2-terminus of the Aβ region in βAPP followed by cleavage at the COOH-terminus of the Aβ region to release the amyloid peptide. These endoproteases produce Aβ that is 40 or 42 amino acids in length. The longer form of Aβ ending in 42 is the dominant form found in the amyloid plaques of AD brains.

Evidence increasingly implicates age, genes (e.g. apolipoprotein E ε4 or α_2-macroglobulin) and inflammation as crucial to the pathological process. In addition, co-morbidities such as cardiovascular and cerebral vascular diseases, or their risk factors, appear also to modulate the expression of the classical histopathological and neurochemical markers of AD, as well as its clinical manifestations. It has been argued for the past decade that the accumulation of

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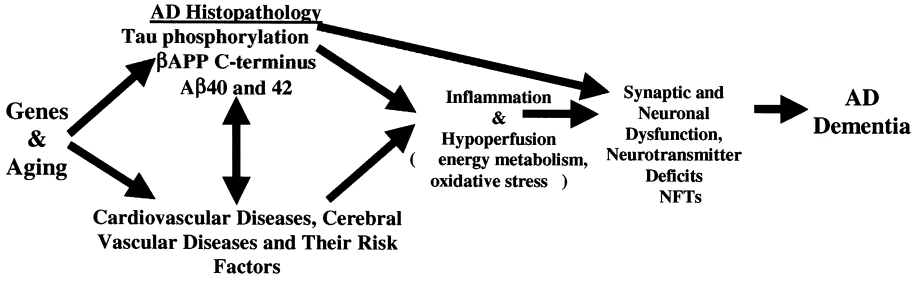

Fig. 1. A scheme of AD pathogenesis reflecting the coalescence of environmental, age-related and genetic factors in the development of AD dementia. A detailed discussion of the hypothesis can be found in [3].

Aβ in the form of fibrillar deposits in the CNS is seminal to the development of AD. Familial cases of AD in which genetic mutations of the genes for βAPP or the presenilin proteins (PS1 and PS2) provide the strongest evidence supporting this conviction. Such mutations lead to increased Aβ production and virtually guarantee the development of AD. However, it remains unresolved how Aβ exerts its toxic effects. Increasing numbers of investigators find that massive accumulations of Aβ can occur in the brains from aged individuals *without* clinical signs of dementia. At face value, these findings suggest that factors in addition to Aβ are needed for the development of AD dementia. This assertion is supported by the failure of transgenic mice overexpressing human βAPP in the CNS to show classical signs of AD pathology (i.e. nerve cell and synaptic loss) despite forming obscene numbers of fibrillar amyloid deposits. One of the distinctive differences found between brains bearing Aβ amyloid from control subjects and the amyloid found in AD brains is markers for inflammation. Thus, sustained brain inflammation might be an essential co-factor in AD pathogenesis.

The evidence for the importance of neuroinflammation in AD rests on several lines of evidence. Over 20 epidemiological studies find that anti-inflammatory drugs like indomethacin and ibuprofen reduce the risk of AD [11–13]. Invariably, the presence of AD dementia coincides with the detection of inflammatory markers around amyloid plaques and dystrophic neurites. These include activated microglia, the resident macrophage of the brain, and activated astrocytes, plus elevation of various cytokines and complement activation products. This spatial interrelationship implies that Aβ may exact inflammatory sequelae. In vitro studies show that Aβ can activate cultured astrocytes [14] and microglia cells [15,16], presumably through Aβ interactions with cell-surface receptors [17–20]. The inflammatory events might also result from Aβ-mediated activation of the complement system in the CNS, an issue on which we shall now focus.

2. The complement system

Complement is a branch of the humoral immune system involved in host defense [21]. Antibody and antibody-independent activation of complement can lead to inflammation, opsonization and cytolysis. Over 20 different proteins make up the major complement pathways, the classical (C1 to C9) and the alternative (C3–C9, factor B, D, P, H) (Fig. 2). The classical pathway typically becomes activated when the first protein in the pathway, C1q of C1, is bound by an antibody or other activator [22]. This initiates a proteolytic cascade, started by the activation of the zymogen serine proteases C1r and C1s, which are bound to C1q. The activation of C1 leads to the sequential activation of C4 and then C3. The alternative pathway differs from the classical pathway in that activation begins at the level of C3 and involves factors B, D, H and P. Proteolytic modification of C3 by either pathway generates the anaphylatoxin C3a, and forms a C3 product that can opsonize. The activation product C3b possesses a newly exposed thioester group that covalently attaches to amines or hydroxyl groups. Once bound, the C3b can act as an opsonin or as a C5 convertase, cleaving C5 to produce the anaphylatoxin C5a and the C5 activation product, C5b. The C5b binds to mem-

branes beginning the formation of the membrane attack complex (MAC) (C5bC6C7C8C9). MACs (C5b–9) damage cells by assembling a lytic pore, composed mainly of multiple C9 proteins, on cell surfaces. The production of the proinflammatory anaphylatoxins, opsonization and MAC-mediated cellular injury all ultimately lead to cytokine-mediated recruitment and activation of immune cells to the site of complement activation.

In addition to the proteins that make up the complement pathways, there are also complement regulatory proteins and complement receptors [23,24]. The regulatory proteins such as C1 inhibitor, clusterin (SP40,40) and CD59 (see discussion below) inhibit complement activation at different stages of the cascade to guard against unremitting complement-induced inflammation. Receptors for anaphylatoxins and activation products of C4 and C3 provide the transducing mechanism mediating immune-cell chemotaxis or activation.

All the components of the classical and alternative complement pathways are found in the central nervous system (CNS) [23,24]. Although the pathogenic role of complement is well recognized in diseases of the periphery [25], the contribution of complement activation to neurodegenerative disorders and diseases is just now being realized [22,24,26]. The activation of complement can be detected in a multitude of human neurodegenerative diseases, e.g. multiple sclerosis (MS), myasthenia gravis (MG), head trauma and stroke, and in animal models of these diseases and disorders (see [22] for review). Blocking complement activation in animal models of MS [27] and MG [28] reduces brain damage, further implicating complement in these degenerative diseases of the nervous system. The expected consequences of prolonged complement activation in the CNS are sustained inflammation resulting in the chronic recruitment and activation of peripheral (e.g. neutrophils) and resident immune cells (e.g. microglia). The effects of such extended inflammation in brains can be seen in transgenic mice chronically expressing inflammatory cytokines in their CNS [29] or rats continuously infused intracerebrally with the inflammatory stimulus lipopolysaccharide [30]. Of particular relevance to AD, both models lead to memory impairment and loss of cholinergic nerve cells in the hippocampus [30,31].

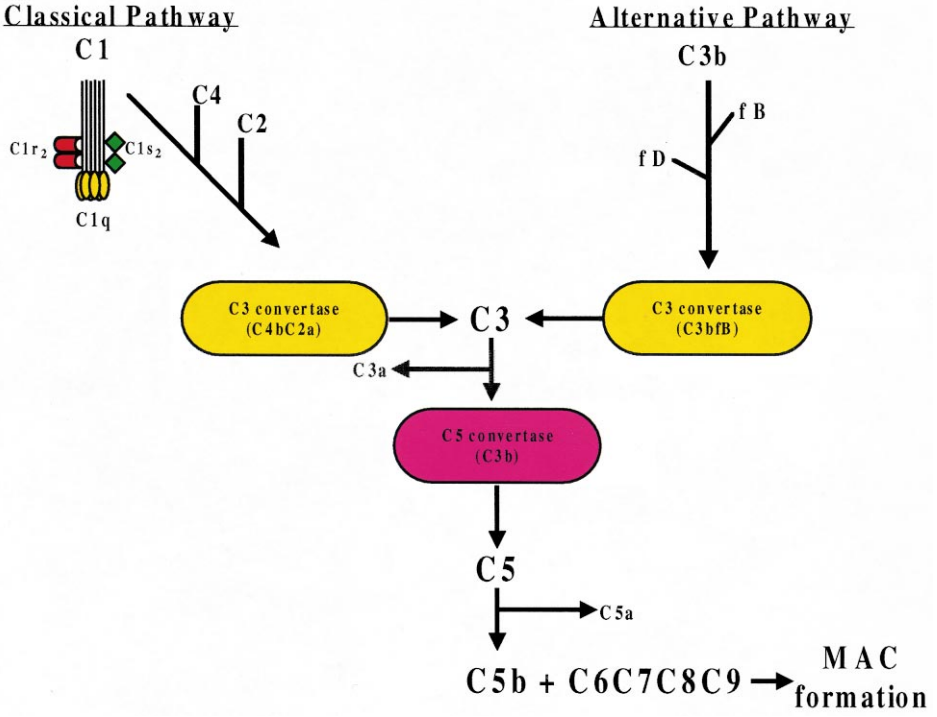

Fig. 2. Diagram of the classical and alternative pathways of complement activation. fB, factor B; and fD, factor D.

3. Complement activation in AD brains and in the brains of βAPP transgenic mice

Although viewed for years as an immune-privileged organ, the central nervous system contains many immune system components. While breach of the blood—brain barrier may allow peripherally produced proteins access to the brain, the role of blood–brain barrier dysfunction in AD remains controversial [18,32–34]. Studies over the last several years, however, have demonstrated that the brain may synthesize immunoglobulins [35] and components of the complement system by both glia and nerve cells [23,24], indicating that leakage across the blood–brain barrier is not necessary for molecular mediators of inflammation to appear in the CNS. The first suggestion that complement proteins may be endogenous to the CNS came with the demonstration that astrocytes in vitro could produce C3 and factor B [36]. Since then, primary astrocyte and astroglioma cultures have been shown to produce all of the activation components of both the classical and alternative pathways [37–41], as well as the terminal complement components [42]. Astrocytes also express receptors for the complement fragments C5a and C3a, also known as anaphylatoxins. The C5a receptor (C5aR) is expressed by astrocytes in the normal brain and is upregulated in inflammatory lesions in vivo and by inflammatory cytokines in vitro [43–45]. In addition, nerve cells also express all of the complement proteins, which also makes them a important source of complement in the CNS.

In addition to astrocytes, microglia, which comprise approximately 10–20% of the brain's cells, produce at least some complement proteins. Because of the difficulty of establishing primary microglial cultures and the paucity of microglial cell lines, less work has been done examining complement protein production by microglia. Microglial cultures have been shown to express the genes for C1q, C2, C3 and C4, [46–51] while human monocyte/macrophage cell lines, used as substitutes for microglia, also express the terminal components [42]. Since microglia are commonly viewed as the brain's resident macrophages. Expression of some complement proteins (e.g. C1q, C1r, C1s, C2, C3, C4, and C9) by microglia has been observed [52–54], but many investigators predict that microglia produce all of the complement components, as do peripheral macrophages [55]. In addition, microglia express complement receptors and regulatory molecules. In fact, identification of microglia often depends on recognition of the complement receptor type 3 (CR3), also known by its components CD11b/CD18 or by the term MAC-1, not to be confused with the membrane attack complex [56,57]. Microglia also express complement receptors type 1 (CR1; CD35) and type 4 (CR4), which, together with CR3, act as phagocytic receptors for complement-fragment opsonized complexes [55,58,59]. These phagocytic receptors are expressed by both resting and activated microglia, although levels may be increased upon activation [60–62]. In contrast, the receptor for C1q, C1qR, is found primarily on process-bearing microglia, indicating constitutive expression [48]. Recently, following the cloning of the C5a receptor, its expression by microglia has been unequivocally demonstrated [44]. Earlier studies showed that C5a produced a chemotactic response from microglia, suggesting that the cells expressed functional anaphylatoxin receptors [63]. The presence of the C3a receptor on microglia has also been demonstrated [64].

Perhaps the most surprising finding is that some complement components are produced by neurons. Both C4 and the B-chain of C1q are produced by neurons in culture, and both C4 and C1qB mRNA and immunoreactivity are detected in neurons in the brain [47,65]. Recent studies have demonstrated that neurons express mRNAs for all of the classical complement proteins as well [66] and that neurons may be the source of much of the complement produced in the brain [67,68]. Neurons may produce CR1, but do not appear to express CR2 or CR3 [69]. Some neuroblastoma cell lines do express C5aR [70] and one study has suggested that C5aR is expressed by neurons in the brain [71]. Another study has shown that neuronal C5aR expression is increased during infection [72]. Neuronal expression of C3aR has not been documented. Thus, neurons in addition to immune cells in the CNS may play an important role in the elaboration of brain complement proteins in neurodegenerative diseases.

Complement regulatory proteins are expressed within the CNS as well. Astrocytes and microglia express the soluble proteins clusterin [42] and C1-inhibitor [73,74]. Neuronal cell lines produce CD59

and membrane co-factor protein (MCP, CD46), but not decay-accelerating factor (DAF, CD55) [75]. Astrocytes or astroglioma cell lines also express the soluble inhibitors factor H, factor I, C4b binding protein, properdin, and S-protein [49,65,76] and the membrane proteins DAF and MCP [76–78]. Constitutive expression of all three membrane-bound complement regulatory proteins, CD46, CD55 and CD59, is very high relative to the expression of the activation pathway components, suggesting that astrocytes are well protected from the adverse effects of complement activation. In contrast, neurons are relatively unprotected from complement attack. Neurons or neuroblastoma cells have been described to express C1-inhibitor and clusterin [65,74,79] and neurons do express the membrane regulator CD59, albeit at low levels relative to astrocytes [80]. Neurons cultured in the absence of glial cells display greatly increased levels of CD59, suggesting that some astrocytic factor causes a downregulation of neuronal CD59 (unpublished observations). Neuronal cell lines also express CD55 and CD46 [81], but only the expression of CD55 by neurons has been documented [82].

4. Complement expression and activation in AD and animal models of AD

Complement proteins were first detected in Alzheimer's disease senile plaques in the early 1980s [83]. Initial studies described the detection of the activation components of the classical pathway, namely C1, C3 and its fragments, and C4 [84–87]. The presence of C3 activation fragments and the lack of soluble components of activation indicated that these complement proteins were bound to the plaque by the process of activation [84]. In contrast, factor B and properdin have not been detected in senile plaques [8], implying that complement activation occurs primarily via the classical pathway. We also have found only activation of the classical complement pathway in the CNS of a rat model of glutamate receptor-mediated neurotoxicity [88]. Moreover, deficiency in factor B, an essential component for the activation of the alternative complement pathway, provides little protection against experimental allergic encephalitis, unlike a C3 deficiency (S.R. Barnum, personal communication). However, it should be noted that activation of one pathway generally leads to the activation of the other, so it is likely that the alternative pathway is activated, but at a very low level.

The presence in AD of the terminal complement components C5b–9, comprising the membrane attack complex, remains controversial. While some investigators cannot detect any of the terminal components in AD brain and detect only low levels of the mRNAs for some of the proteins [2,86,87], numerous other investigators have consistently demonstrated the presence of a neoepitope of the MAC [8,89,90]. Typically, the MAC is not detected in senile plaques, but rather on dystrophic neurites and neurofibrillary tangles adjacent to the plaques. These results suggest that complement activation occurs at the site of the senile plaque, but bystander attack produces MAC formation and insertion in adjacent neurons. Ultrastructural studies have shown the presence of MAC immunoreactivity associated with neuronal lysosomes and cytoskeletal proteins, suggesting that neurons attempt to defend themselves against complement attack by internalization of the MAC [89]. Interestingly, non-demented individuals occasionally present at autopsy with dense-core Aβ plaques and neurofibrillary tangles in most regards indistinguishable from those of AD patients, and, in fact, such neuropathological changes may not be as uncommon as previously thought. However, as opposed to the AD patients, these controls displayed no terminal complement components around plaques or tangles [91]. These studies imply that the chronic inflammation, of which complement activation is a part, may be at least partially responsible for the progression of a patient to a state of dementia.

Besides the dense senile plaque deposits of Aβ, the AD brain often contains deposits of Aβ, referred to as cerebrovascular Aβ, or congophilic angiopathy, around blood vessels [92]. As with the plaques, complement proteins were found associated with the cerebrovascular Aβ [93,94]. Pericytes, phagocytic cells that exist in very close relationship to endothelial cells in the cerebral microvasculature, produce C1q as well as Aβ in culture [95]. Pericytes, therefore, may be one source of inflammatory molecules associated with cerebrovascular amyloid.

Recent studies have suggested that classical path-

way components may be greatly upregulated in AD. The levels of mRNAs for the classical pathway proteins are increased in AD brain compared to control [68], and in fact may be increased to levels higher than that seen in peripheral organs. The largest increases occur in those areas of the brain displaying the greatest pathology, such as the cortex. Areas of the brain displaying little pathology, such as the cerebellum, display small or no increases in complement mRNA levels. Levels of complement proteins were also increased, in addition to the mRNAs. For example, increased levels of C1q protein were found in AD cortex, but not in AD cerebellum [96].

The Alzheimer brain also contains diffuse plaques, containing non-filamentous Aβ, in many regions of the brain, including those generally not affected by the disease, such as the cerebellum. Unlike the senile plaque, only the very earliest steps of complement activation appear to occur in diffuse or primitive plaques. For example, the C4 activation fragment, C4d, was detected in diffuse plaques, while C3d, a fragment resulting from the next step in the activation pathway, was not [97]. The MAC was not detected in diffuse plaques either. The lack of complete complement activation by diffuse plaques may account, at least in part, for the relative sparing of brain areas containing only the diffuse deposits.

Multiple complement proteins have been detected in cerebrospinal fluid (CSF). Some, such as C1q, C3, C4, C9, CD59, and clusterin are normally at detectable levels in CSF [98–100]. In AD CSF, the levels of clusterin are either elevated [101] or unchanged [102] from control levels. C1q levels, conversely, are dramatically decreased in AD CSF compared to control, and the decline in C1q correlates to the severity of the disease [103]. Levels of other complement proteins have been found to change in CSF with age, but there is apparently no difference between aged controls and aged AD patients [104].

The Alzheimer brain apparently mounts a defense against chronic complement attack. Levels of complement regulatory proteins, such as clusterin [105], C1-inhibitor [74,84], lactoferrin [94] and C4 binding protein [106] are all upregulated in AD. In addition to higher levels being found in the AD brain, C1-inhibitor is detected in its cleaved form in AD [74]. Presence of the cleaved form, therefore, indicates that C1 has been activated and then inhibited by the C1-inhibitor. Immunostaining for CD59, which is detected most strongly in endothelial cells in the normal brain, in the AD brain predominates in the same areas that stain for the MAC: dystrophic neurites and neurofibrillary tangles [107]. Finally, neurons apparently internalize the MAC in an attempt to limit cell damage (see above).

The βAPP gene is contained on human chromosome 21, which is trisomic in Down's syndrome. Most people with Down's syndrome who live the past 40 years of age develop both the neuropathological changes and the cognitive deficits seen in Alzheimer's disease [108,109], supporting the hypothesis that the βAPP gene is important in the initiation or progression of AD. Consistent with the findings in AD brain, complement immunoreactivity was detected in association with dense core plaques in the brains of older Down's patients, but not with the amorphous, diffuse deposits of Aβ seen in younger Down's brains [110]. There is also a sequential order to the appearance of complement proteins in relation to amyloid plaques. C1q, C3 and C4 have been detected in early plaques whereas MAC appears later [110], possibly coinciding with the onset of dementia.

Within the past several years, research into the causes and possible treatment for AD has been advanced by the development of several different transgenic mouse models. Mice have been developed and bred that are transgenic for human βAPP, both in its normal form [111,112] and containing familial AD producing mutations [113,114]. In addition, transgenic lines have been developed that express the familial AD producing forms of another protein, presenilin 1 (PS) [115–117]. In general, mice overexpressing the normal human form of βAPP display little AD-like pathology, although some cognitive deficits have been described [111,118,119]. In contrast, several lines of mice overexpressing familial AD forms of βAPP develop Aβ plaques [113,120] and display age-related impairments in learning and memory [120]. Very recent studies have described signs of inflammation, including complement proteins, associated with the plaques in these animals [121,122]. These inflammatory markers increased with age. However, complement proteins were not found contiguous with all or even most plaques in these mice. When care was taken to cross PS-βAPP double transgenic mice onto a complement sufficient mouse

strain, reactive microglia were found to surround the Aβ plaques, whereas few microglia were seen in the mouse strains that express low levels of complement [123]. Also, Aβ activates mouse complement less efficiently than human complement [124], and complement becomes associated with the plaques via complement activation (see above). Taken together, these results suggest that complement activation occurs in mice transgenic for human Alzheimer's disease-related proteins as long as the background strain of mouse used is complement sufficient. In addition, a mouse strain that is doubly (or triply) transgenic for the AD-related proteins and human C1q may provide a better in vivo model for Aβ-mediated complement activation.

5. β-Amyloid peptide interactions with complement proteins

Rogers and colleagues reported the first direct evidence that aggregated Aβ alone activates complement [125]. Aβ absorbed to the plastic of microtiter plates supports the activation of complement as indicated by the deposition of the activation product C3d on the wells. Since then, numerous laboratories have replicated this finding and shown that Aβ activation of complement can also occur in the fluid phase by the classical and alternative pathways [126–129]. This activation results in the production of anaphylatoxins C3a [126,130] and C5a [128], the opsonization of Aβ by the covalent addition of C3 [126–128] and the formation of the cytolytic MAC [90]. In our studies, the formation of C3 activation products, C3a and C3b, is a direct consequence of the assembly of the classical C3 convertase (C4bC2a) on the aggregated amyloid [130].

Fibrillar Aβ activates complement readily, whereas non-fibrillar peptide does not [126–132]. The aggregation of peptide through natural aging [128,132] or by forced fibril formation at pH 6.0 [126] both support complement activation. However, on a molar basis, aggregated Aβ1–42 activates complement more potently than aggregated Aβ1–40 [126,131]. This difference may reflect the ability of Aβ1–42 to form fibrils with β-sheet conformation more readily and at lower concentrations than Aβ1–40 [133]. It should also be noted that Aβ interactions with C1q

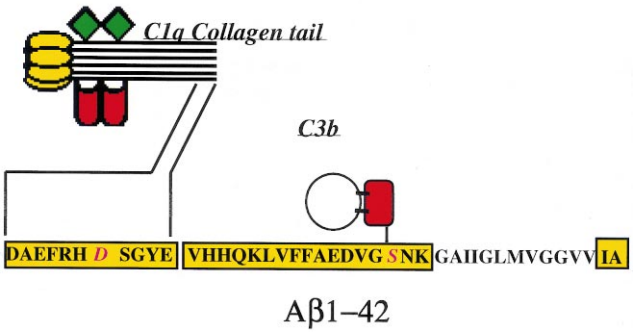

Fig. 3. Proposed sites of interaction between complement proteins C1q and C3 with the Aβ1–42. Amino acids in italics, Asp^{7} and Ser^{26}, are suspected of being important for the binding of C1q collagen tail and C3, respectively.

leads to increased amyloid aggregation [134–136]. This interaction may be significant since C1q is often found associated with incipient amyloid deposits [110].

Aβ-mediated activation of the classical pathway results from interactions between residues 14–26 of the A chain of the collagen-like tail of C1q [131] and the first 11 residues of Aβ [125,126,137] (Fig. 3). The binding interaction between C1q and Aβ may be mainly ionic since the first 11 residues of Aβ are predominantly anionic while those of the C1q A chain residues 14–26 are cationic [137]. Consumption of C4 in human serum requires the presence of the first 11 amino acids of Aβ, and modification of the aspartic acid at residue 7 by isomerization or by substitution with asparagine reduces C4 consumption [138]. We find that the C1q binding to aggregated Aβ is reduced if the NH_2-terminus of Aβ is truncated by two amino acids (Aβ3–42) and is virtually eliminated when using aggregated Aβ12–42 (unpublished observation). The rat sequence of Aβ1–42 which differs from the human sequences at three residues within the first 16 amino acids also fails to bind C1q (unpublished observation). Interestingly, all the Aβ fragments that failed to bind C1q still activate C3 in human serum (unpublished observation), presumably through the alternative (C1q independent) complement pathway. This also implies that C3 can be activated by and binds to residues in the Aβ12–42 sequence. We have observed that C3 can bind to aggregated Aβ1–42 and covalently modify the peptide even in the absence of complement activation [126]. This latter binding interaction appears to be

reasonably high affinity. Studies on the displacement of 0.015 μM iodinated apolipoprotein E binding to fibrillar Aβ1–40 reveal that C3 has a 50% inhibitory (IC_{50}) value of 0.085 μM compared to that of 0.18 μM for C4 and >1 μM for C1q (H. LeVine, unpublished observation). These binding results suggest that C3 and C1q bind to different sites on Aβ. However, there may be some overlap between the binding sites for C1q and C3 on amyloid fibrils. The level of C1q binding to aggregated Aβ1–42 increases several fold upon depletion of C3 from human serum (unpublished observation). The increased binding of C1q to Aβ can be reduced upon restoring C3 to the depleted serum (unpublished observation). Recently, Bradt and Cooper showed that the Aβ residues 12 through 28 provide an activation/binding site for C3 [139]. The serine at position 27 of Aβ1–42 provides a likely point for covalent attachment for C3b opsonization (Fig. 3). Thus, any competition between the binding of C3 and C1q might be explained by the possible apposition of their two binding sites on Aβ (see Fig. 3).

Complement activation can lead to cell death in nerve cell cultures. The addition of human serum to mixed rat hippocampal cell cultures results in heterologous complement attack leading to nerve cell death. The addition of as little as 0.8 μM Aβ1–40 to human serum exacerbates the cell loss more than treatment with 50 μM Aβ alone [140]. Similar results have been obtained with human nerve cell lines [141,142]. Moreover, the treatment of the SH-SY5Y human neuronal cell line with Aβ stimulates complement production by these cells, which then results in MAC formation [143].

We have employed cultured dog leptomeningeal smooth muscle cells to examine further the consequences of Aβ-mediated complement activation on cell viability [144]. This system is unique in that cultured smooth muscle cells treated with 10 μM Aβ1–42 form a calyx of amyloid fibrils on their cell surfaces and within several days undergo programmed cell death (apoptosis) [145–147]. Addition of human serum to Aβ-embedded smooth muscle cells results in complement activation involving the binding of C1q to the Aβ, the activation of C3 and the formation of MACs. Activation products of C3 and the MACs can be visualized immunocytochemically in association with the amyloid laden cells. In this instance, the combination of complement activation and Aβ does not increase cell death. Contrary to expectations, treating the cultures with serum reduces the amount of amyloid-induced toxicity despite the activation of complement (unpublished observation). Although surviving the induced complement attack may be of immediate benefit to the smooth muscle cell, it should be borne in mind that the amyloid-embedded smooth muscle cells now become a potential nidus of chronic inflammation. It remains to be determined if such a scenario is played out in vivo, but it merits noting that complement activation occurs in the cerebral vascular amyloid found in the leptomeningies of AD brains [94]. It may be speculated that the anaphylatoxins produced as the result of complement activation increase blood–brain barrier permeability [148–151] and vasomotor activity, events that could also locally compromise cerebral perfusion.

6. Preventing complement activation in Alzheimer's brains

Several animal models of chronic neurodegeneration benefit from complement inhibition. Morgan and colleagues [27,28,152] have shown in a rodent model of experimental allergic encephalitis (EAE) soluble CR1, a cell surface inhibitor of complement activation that binds activated C4 and C3, protects animals from the development of MS-like symptoms. Recently, Barnum and co-workers also reported that overexpressing *crry*, another protein that inhibits complement by binding activated C4 and C3, in mouse brain also protects against EAE [153]. These results provide some of the first indications that inhibiting complement activation has application to the treatment of neurodegenerative disease. Tests in animal models of AD or AD patients have yet to be done to determine if inhibiting complement activation exclusively will be beneficial. However, several studies now show that conventional and non-conventional means might be employed to prevent Aβ activation of complement [22].

In vitro studies have already begun to address how to prevent Aβ-mediated complement activation. Activation resulting from aggregated Aβ1–42 can be prevented by inhibitors of the serine proteases C1r

and C1s [126,130]. FUT-175, a potent inhibitor of C1r and C1s as well as a number of other serine proteases [154], prevents Aβ activation of complement via the classical pathway. This inhibition does not prevent C1q from binding to Aβ, but it does drastically diminish the amount of C3 activation. Similarly, C1 inhibitor, a natural protein inhibitor of C1r and C1s found in serum and tissue, also inhibits Aβ activation of complement [126]. Both compounds also block complement activation in the Aβ1–42 treated smooth muscle cell cultures [144]. Recent attempts to improve the selectivity and potency of inhibitors of C1r and C1s have focused on a series of benzoxazinone compounds [155–157]. Currently, only a small number of benzoxazinones have been tested, but they are effective in blocking Aβ-mediated complement activation in vitro [22] or in smooth muscle cell cultures (unpublished observation). Vaccinia virus complement control protein, which inhibits by binding activated C4 and C3, has also been recently reported to prevent in vitro complement activation by Aβ [129]. Thus, multiple opportunities exist along the complement pathways to intervene in its activation by Aβ.

An alternate strategy is to target Aβ itself as a way of preventing complement activation. This can be accomplished by small molecules binding to the Aβ region needed for the interaction with C1q (Aβ1–11) or C3 (Aβ12–28), or blocking the interaction of Aβ with its binding site on C1q [131,158]. Congo red, a histochemical stain for amyloids, blocks the binding of C3 to Aβ1–42 fibrils in vitro [130] and amyloid fibrillized on smooth muscle cells in culture [127]. No effect on C1q binding to Aβ was detected in the presence of Congo red. Interestingly, another stain for amyloids, thioflavine S, had no such effect on the binding of complement proteins to aggregated Aβ1–42 (unpublished observation). Efforts have also focused on blocking Aβ interactions with C1q by competition with peptides that bear the same sequence as that region of C1q to which Aβ binds [158].

Although none of the aforementioned approaches have reached clinical trials in AD patients, anti-inflammatory drugs have. The steroid prednisone has a number of anti-inflammatory effects [159]. Clinical trials in AD patients have been initiated with this steroid, using a prednisone dose (10 mg/day) reported to decrease serum levels of C3. However, results from this study failed to show a benefit of prednisone compared to the placebo group in a year-long study [160].

Hoechst-Marion-Roussel has been developing propentofylline for the treatment of AD. Propentofylline is a xanthine derivative that acts as a cAMP/cGMP phosphodiesterase inhibitor as well as increases the extracellular concentration of adenosine by blocking its cellular reuptake [161]. In vivo treatment with propentofylline abates microglia activation and associated damage in animal models of neurodegeneration [162] and in cell culture studies [163], suggesting that propentofylline has anti-inflammatory activities. However, it remains to be determined what, if any, effect this compound has on complement expression, although it reportedly reduces expression of tumor necrosis factor α and other cytokines by cultured cells [164]. Propentofylline has been shown to have beneficial effects in a 52-week clinical trial in AD patients [165].

Recently, two new NSAIDs, celecoxib (Searle/Monsanto) and rofecoxib (Merck), that specifically inhibit cyclooxygenase-2 (COX-2) are being developed for the treatment of arthritis. Both compounds are also under investigation for the treatment or prevention of AD [166,167]. Several laboratories report that anti-inflammatory drugs effectively block neurotoxicity resulting from chronic, low-level brain inflammation [168] or by Aβ in cell cultures [169,170]. It remains to be determined how the new COX-2 inhibitors or other anti-inflammatory drugs affect complement-mediated inflammation.

7. Conclusions and commentary

Numerous epidemiological studies support intervening or preventing AD by blocking inflammatory reactions that develop in the course of the disease. Histopathological studies of AD brains suggest that reactive microglia in association with amyloid deposits are the possible source of this inflammation. Recent in vitro studies implicate complement activation by aggregated Aβ as a potential initiator of chronic inflammation through sustained glial cell activation, since both astrocytes and microglia bear receptors for the anaphylatoxins C3a and C5a. The anaphyla-

toxins might also affect blood flow to those brain regions exhibiting Aβ-mediated complement activation because C3a and to a greater extent C5a are vasoactive. Moreover, the opsonization of amyloid plaques by activated C3 and anaphylatoxin production would, through chemotaxis, target microglial attacks on those regions of AD brains with the greatest numbers of amyloid deposits. Finally, this localized complement activation leads to the formation of MACs, which compromise the physiological integrity of nerve cells upon inserting into their cell membranes.

Presently, evidence from AβPP transgenic mice and from non-demented patients with amyloid plaques suggest that fibrillar Aβ alone may not be sufficient to initiate brain inflammation. It may be that other factors such as an age-related increase in steady-state levels of brain inflammation [171], or severe or subtle traumatic brain injury, resulting from blunt trauma or cardiovascular disease, might initiate inflammation that is exacerbated by the presence of amyloid plaques. This may explain why brain injury is more extensive in βAPP transgenic mice than their control counterparts [172–175]. In this context, it may be more appropriate to think of amyloid deposits as performing the same role as 'oily rags' in initiating a conflagration. However, in the case of AD, the conflagration is replaced by an unremitting, localized low-level inflammation.

NSAIDs provide a promising, but largely untested, approach to blocking the consequences of Aβ-mediated inflammation. A small clinical trial indicates that the COX inhibitor, indomethacin, might have a beneficial clinical effect in AD patients [176]. However, specific COX-2 inhibitors may provide the long-term safety that is expected for chronic treatment or prevention of AD brain inflammation. Efforts are also directed toward preventing complement activation by Aβ from occurring by blocking the interaction between complement proteins and Aβ, or inhibiting the proteolytic cascade that leads to complement activation. One can also envision that the elimination of amyloid plaques through blocking Aβ production or its deposition will also diminish inflammation in AD brains. However, which of these complement-directed approaches will prove the most successful in treating AD or preventing the dementia remains to be determined.

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